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Amendment and Response

Serial No.: 09/180,340

Confirmation No.: 6674

Filed: August 20, 1999

For: STABLE RECOMBINANT YEASTS FOR FERMENTING XYLOSE TO ETHANOL

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Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1-16. Canceled

17. (Currently Amended) A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising:

(a) transforming the cells with a replicative and integrative plasmid comprising an autonomous replicating sequence, exogenous DNA, and a first selection marker; and

(b) repeatedly replicating the cells from step (a) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, promoting the retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA, and wherein the method further includes the step of repeatedly replicating the progeny cells from step (b) to produce a number of generations of progeny cells in the absence of selection for cells which include the selection marker, so as to promote the loss of the plasmid in subsequent generations of progeny cells and recovering [yeast] the cells each containing multiple copies of the exogenous DNA integrated into its chromosomal DNA, and [The method of claim 16] wherein the cells are yeast cells and the exogenous DNA includes genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, which also serve as the first selection marker.

18-28. Canceled

29. (Previously Presented) A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA including genes encoding xylose reductase, xylitol

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homologous to a reiterated DNA sequence of a target yeast cell, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of the target yeast to form stable integrants which ferment xylose to ethanol.

30-34. Canceled

35. (New) The method of claim 17, wherein the plasmid also includes a second selection marker for selecting cells which include the plasmid.